CONDITIONAL PETITION FOR EXTENSION OF TIME

If any extension of time for this response is required, Applicants request that this be considered a petition therefore. Please charge the required fee to Deposit Account No. 14-1263.

ADDITIONAL FEES

Please charge any further insufficiency of fees, or credit any excess to Deposit Account No. 14-1263.

REMARKS

Claims 22-26 were pending in the application. In accordance with Examiner's requirement to more clearly reflect the elected subjected matter, claims 23-26 have been canceled, and claim 22 has been amended. New claims 27-36 have been added.

Amendments to the claims are attached herewith.

Claim 22 is now directed toward a diagnostic kit. The kit, originally claimed in claim 12, is encompassed by the elected subject matter. Support for claim limitations 22(b) and 22(c) can be found on pages 3/3rd ¶, and 7/2nd ¶.

In addition, new claims encompassing vectors and cells harboring the vectors are also believed to be encompassed by the elected subject matter. See Restriction Requirement, paper 10.

In the Specification

An amended sequence listing in paper and CRF, is filed herewith.

This amendment necessitated amending pages in the specification where the newly identified and listed sequences are disclosed. The amendment to the specification is attached herewith.

In brief, both marked up and clean copies of substitution pages 13-15 are attached.

CONCLUSION

It is believed in good faith that none of the amendments or new claims add new matter.

Further, it is believed that all issues raised by the Examiner have been satisfactorily addressed.

Applicants respectfully suggest that the claims are now in condition for allowance, and that allowance be granted.

Respectfully Submitted,

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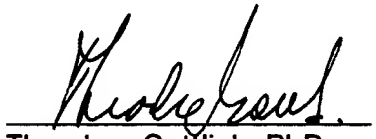

Theodore Gottlieb, PhD
Reg. Nr. 42,597

AMENDMENT TO THE CLAIMS

IN THE CLAIMS

1.- 21. (Canceled)

22. (Currently amended) A ~~method~~ kit comprising at least one diagnostic polynucleotide, wherein the at least one diagnostic polynucleotide is selected from the group consisting of:

(a) a polynucleotide according to SEQ ID NO: 2;

(b) a polynucleotide that is at least 80% identical to the polynucleotide of SEQ ID NO: 2, and encodes a G-protein-coupled receptor EDG6 ;

(c) a polynucleotide encoding a G-protein-coupled receptor EDG6 having at least 82% sequence identity to, or, 91% sequence similarity with, the amino acid sequence of SEQ ID NO: 1;

(d) a polynucleotide complementary to either of the polynucleotides of (a),(b) or (c);

e) a polynucleotide consisting of a portion of either of the polynucleotides of (a), (b), (c) or (d), and,

wherein the kit is suitable for detecting the presence of leukocytes and lymphoid cells in a tissue or cell sample obtained from a human subject.

~~for determining the presence of leukocytes in a cell and/or tissue sample taken from a living subject, said method comprising the steps of:~~

~~(a) obtaining said sample from said subject,~~
~~(b) extracting ribonucleic acid (RNA) from said cell and/or tissue sample, and (c) performing a Northern blot analysis of said RNA,~~

~~wherein detecting RNA having sequences complementary to the DNA sequence~~

~~described by SEQ ID NO: 2 and/or at least one portion thereof, in said Northern blot analysis, is indicative of the presence of leukocytes in said anatomical sample.~~

23. - 26. (Canceled).

27. (New) The kit of claim 22, wherein the at least one diagnostic polynucleotide is suitable for use as a probe in blotting assays, wherein hybridization of the probe to immobilized nucleic acid sequences obtained from a tissue- or cell-sample is indicative of the presence of leukocytes or lymphoid cells in the sample.

28.(New) The kit of claim 22, wherein the at least one diagnostic polynucleotide is suitable as a primer for performing PCR on the tissue- or cell-sample's RNA or DNA, and wherein the produce of said PCR is indicative of the presence of leukocytes or lymphoid cells in the sample.

29. (New) The kit of claim 22, wherein the polynucleotide having at least 80% identity with SEQ ID NO: 2, has the sequence of SEQ ID NO: 4.

30. (New) The kit of claim 22, wherein the polynucleotide encoding a G-protein-coupled receptor EDG6 having at least 82% sequence identity to, or, 91% sequence similarity with, the amino acid sequence of SEQ ID NO: 1, has the sequence of SEQ ID NO: 4.

31. (New) A vector suitable for cloning and gene transfer, the vector comprising a promotor operably linked to either,

(a) a polynucleotide according to SEQ ID NO: 2;

(b) a polynucleotide that is at least 80% identical to the polynucleotide of SEQ ID NO: 2, and encodes a G-protein-coupled receptor EDG6 ;

(c) a polynucleotide encoding a G-protein-coupled receptor EDG6 having at least 82% sequence identity to, or, 91% sequence similarity with, the amino acid sequence of SEQ ID NO: 1;

(d) a polynucleotide complementary to either of the polynucleotides of

(a),(b) or (c),

wherein the promoter effectively provides for the expression of the polynucleotide of (a), (b), (c) or (d) in a mammalian host cell.

32. (New) A bacterial cell comprising the vector of claim 31.

33. (New) A mammalian cell comprising the vector of claim 31.

34. (New) The mammalian cell of claim 32, wherein the cell is a human cell.

35. (New) The vector of claim 31, wherein the promotor is a eukaryotic viral promotor.

36. (New) The kit of claim 22, further comprising one or more monoclonal antibodies directed to a protein having the amino acid sequence of SEQ ID NO: 1.

IN THE SPECIFICATION

Replace pages 13-15 currently of record with the following substitute pages.

GT-3') (SEQ ID NO:5) , R3 (5'-CG-GGA-TCC-GAA-RGY-RTA-SAD-SAD-RGG-RTT-3') (SEQ ID NO:6) . Cycle: 94°C, 60 sec.; 48-63°C, 30 sec.; 72°C, 90 sec.; 35 cycles. For the amplification of the 3' and 5' ends of the human edg6 cDNA, a RACE-PCR was carried out with the following primers: 5'hGSPRT (5'-TTG-GAG-CCA-AAG-ACG-TCG-GCC-3') (SEQ ID NO:7) , 5'-HGSP1 (5'-AGG-CAG-AAG-AGG-ATG-TAG-CGC-3') (SEQ ID NO:8) , 5'-HGSP2 (5'-GCG-CTC-CCC-TGC-AGT-GAA-GAG-3') (SEQ ID NO:9), 3'-hGSP1 (5'AGT-GAC-CTG-CTC-ACG-GGC-GCG-3') (SEQ ID NO:10) , 3'hGSP2 (5'CTCTTCACTGCAGGGGAG-CGC-3') (SEQ ID NO:11) . The reactions were carried out according to the protocol of M.A. Frohman (Frohman, 1995). The amplification of the 5' end of the murine edg6 cDNA was likewise done with the help of the RACE-PCR with the following primers: 5'-mGSPRT (5'-CTC-ACC-TCG-TCT-GGG-AGG-GCC-TGC-3') (SEQ ID NO:12) , 5'-mGSP1 (5'-TGG-GCA-ACT-GGC-TGG-TCC-AAG-CTC-3') (SEQ ID NO:13) , 5'-mGSP2 (5'-GCC-TCG-GGC-CCA-GAT-CCT-CCA-GGG-GTG-CTG-CGG-ACG-CTG-GAA-ATG-CTG-G-3') (SEQ ID NO:14). Before, as described above, a reverse transcription was done with 10 µg of overall RNA of the murine cell line 18. The 5'-mGSP2 primer contains a part of the myc-epitope sequence for further experiments. The primers were selected on the basis of the murine EST sequence of the cDNA clone va16c04.r1 (Gene Bank entry no. AA254425), which has a high homology with the 3' end of the coding human edg6 cDNA. The reactions were also carried out according to the protocol of M.A. Frohman (Frohman, 1995) with an additional cleaning step by means of "MicroSpin S-400 HR" columns of the firm of Pharmacia Biotech on the basis of the supplied protocol following the 5' polyadenylation reaction. Further, 10 µl of undiluted presentation DNA were used for both amplifications of the RACE-PCR. The murine edg6 cDNA fragment, which was used in the Northern blot as a radioactively marked sample, was amplified as described above through the reverse transcriptase polymerase chain reaction from an overall RNA preparation of the murine

cell line 18 with 25 pmol each of the 3' primer (5'-CCA-CGT-CCT-CCT-GCC-CGC-CGC-3') (SEQ ID NO:15) and 25 pmol of the 5'-mGSP2 primer (see above). Cycle: 94°C, 60 sec.; 50°C, 60 sec.; 72°C, 90 sec.; 35 cycles. The amplification of the genomic 3' sequence of the human edg6 was done by means of PCR from 400 ng HEp2 genomic DNA with 25 pmol of the 3'-hGSP2 primer (see above) and 25 pmol of the CA primer (5'-CCA-CTT-CCC-GCA-ACG-CCC-AGA-3') (SEQ ID NO:16). Cycle: initial denaturing, 95°C, 5 min.; 95°C, 30 sec.; 60°C, 30 sec.; 72°C, 90 sec.; 30 cycles.

Cloning and sequencing

The cDNA fragments of the PCR reactions with the degenerated primers were cloned into the pZErO-2 vector of the firm of Invitrogen to Bam HI Verdau. The human edg6 RACE-PCR products were cloned into the same vector to HIND III/Pst I Verdau. They were ligated to a full-length clone at the Pst I interface. The murine edg6 5'-RACE-PCR product was cloned into the pZErO-2 vector to HIND III/Eco RV restriction. For this, the RACE-PCR product was HIND III-digested after a T4-polymerase reaction. The human cDNA fragment for the radioactive marking was isolated to Pst I/Aat II-restriction of the full-length clone (bp 438-842). The amplified murine cDNA fragment (bp 328-637) was cloned into the Apa I cut pZErO-2 vector. This fragment was used as a sensor in Northern blot after radioactive marking. All the fragments were sequenced with the "Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP" of the firm of Amersham International and analysed with the help of the Li-Cor sequencer of the firm of MWG Biotech according to the supplied protocols.

Construction, expression and FACS analysis of the myc-epitope marked human EDG6 receptor

The construction of the C-terminal myc-epitope marked human EDG6 receptor and its expression in HEK293 cells and its analysis by means of throughflow cytometry was done as described (Emrich et al., 1993).

Computer analyses

Sequence comparisons, database research and statistical calculations were done with the help of HUSAR package V4.0 at the German Cancer Research Centre in Heidelberg and with the ClustalX V1.62b PC programme.

GT-3') (SEQ ID NO:5) , R3 (5'-CG-GGA-TCC-GAA-RGY-RTA-SAD-SAD-RGG-RTT-3')(SEQ ID NO:6) . Cycle: 94°C, 60 sec.; 48-63°C, 30 sec.; 72°C, 90 sec.; 35 cycles. For the amplification of the 3' and 5' ends of the human edg6 cDNA, a RACE-PCR was carried out with the following primers: 5'hGSPRT (5'-TTG-GAG-CCA-AAG-ACG-TCG-GCC-3') (SEQ ID NO:7) , 5'-HGSP1 (5'-AGG-CAG-AAG-AGG-ATG-TAG-CGC-3') (SEQ ID NO:8) , 5'-HGSP2 (5'-GCG-CTC-CCC-TGC-AGT-GAA-GAG-3')(SEQ ID NO:9), 3'-hGSP1 (5'AGT-GAC-CTG-CTC-ACG-GGC-GCG-3') (SEQ ID NO:10) , 3'hGSP2 (5'CTCTTCACTGCAGGGGAG-CGC-3') (SEQ ID NO:11) . The reactions were carried out according to the protocol of M.A. Frohman (Frohman, 1995). The amplification of the 5' end of the murine edg6 cDNA was likewise done with the help of the RACE-PCR with the following primers: 5'-mGSPRT (5'-CTC-ACC-TCG-TCT-GGG-AGG-GCC-TGC-3') (SEQ ID NO:12) , 5'-mGSP1 (5'-TGG-GCA-ACT-GGC-TGG-TCC-AAG-CTC-3') (SEQ ID NO:13) , 5'-mGSP2 (5'-GCC-TCG-GGC-CCA-GAT-CCT-CCA-GGG-GTG-CTG-CGG-ACG-CTG-GAA-ATG-CTG-G-3') (SEQ ID NO:14). Before, as described above, a reverse transcription was done with 10 µg of overall RNA of the murine cell line 18. The 5'-mGSP2 primer contains a part of the myc-epitope sequence for further experiments. The primers were selected on the basis of the murine EST sequence of the cDNA clone va16c04.r1 (Gene Bank entry no. AA254425), which has a high homology with the 3' end of the coding human edg6 cDNA. The reactions were also carried out according to the protocol of M.A. Frohman (Frohman, 1995) with an additional cleaning step by means of "MicroSpin S-400 HR" columns of the firm of Pharmacia Biotech on the basis of the supplied protocol following the 5' polyadenylation reaction. Further, 10 µl of undiluted presentation DNA were used for both amplifications of the RACE-PCR. The murine edg6 cDNA fragment, which was used in the Northern blot as a radioactively marked sample, was amplified as described above through the reverse transcriptase polymerase chain reaction from an overall RNA preparation of the murine cell line 18 with 25 pmol each of the 3' primer (5'-CCA-CGT-CCT-CCT-GCC-CGC-CGC-3') (SEQ ID NO:15) and 25 pmol of the 5'-mGSP2 primer (see above). Cycle: 94°C, 60 sec.; 50°C, 60 sec.; 72°C,

90 sec.; 35 cycles. The amplification of the genomic 3' sequence of the human edg6 was done by means of PCR from 400 ng HEp2 genomic DNA with 25 pmol of the 3'-hGSP2 primer (see above) and 25 pmol of the CA primer (5'-CCA-CTT-CCC-GCA-ACG-CCC-AGA-3') (SEQ ID NO:16). Cycle: initial denaturing, 95°C, 5 min.; 95°C, 30 sec.; 60°C, 30 sec.; 72°C, 90 sec.; 30 cycles.

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